

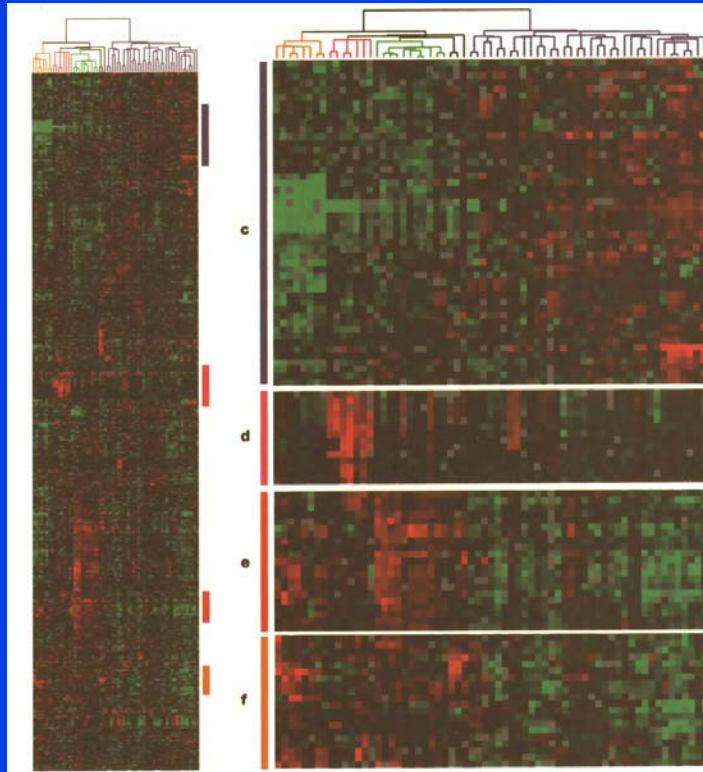
NIBIB Workshop: Emerging Technologies and Applications

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Genomics and Proteomics: Translation into Molecular Medicine

Cluster Analysis of Gene Expression (Gene Chip) in Clinical Breast Cancer

Relative Expression of Single Genes

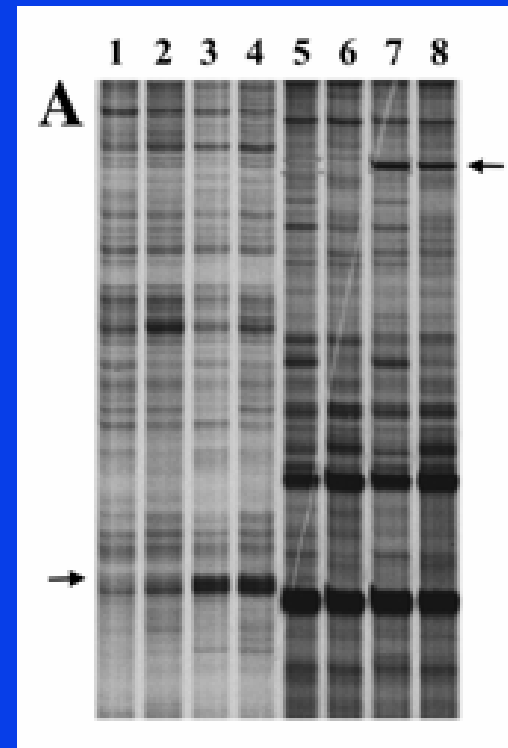


Breast Cancer Samples

Nature 406:747-752, 2000

Differential Display of Expressed Genes in Drug Resistant Breast Cancer Cells

Amplified Transcripts of Single Genes



Breast Cancer Cells

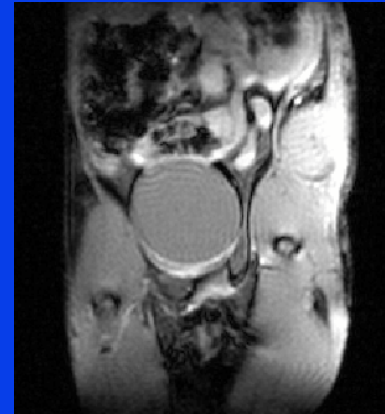
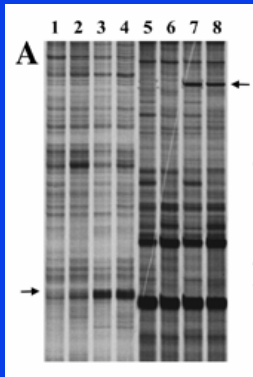
Cancer Research 61:6540-6547, 2001

New Linkages in Medical Imaging

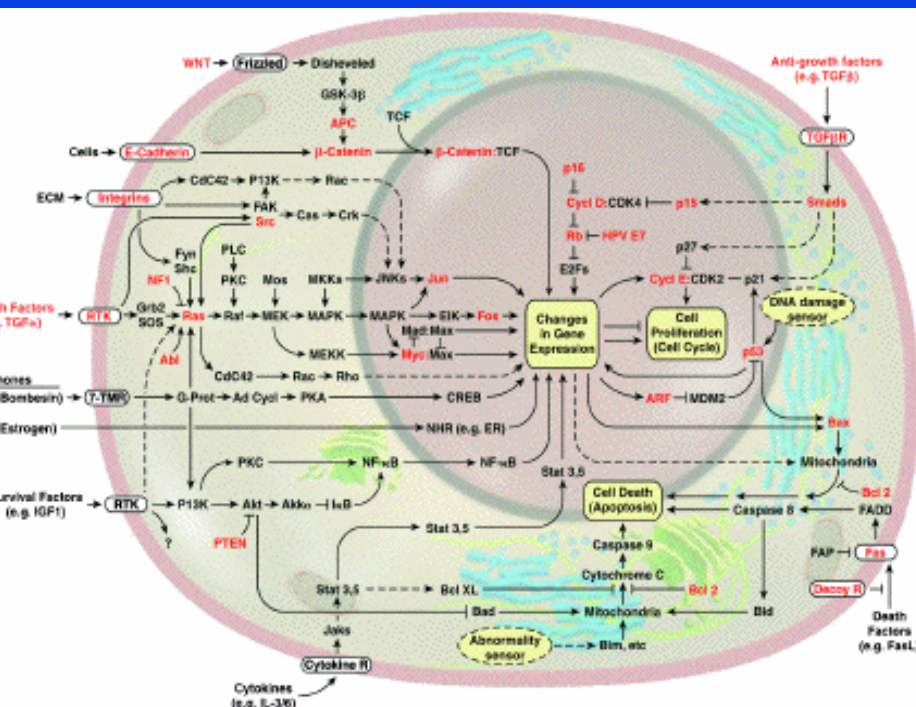
Molecular
Biology

Radiological
Sciences

Molecular
Imaging



Molecular Targets in Cell Biology



Cell 100, 57–70, 2000

Gene Expression in Cancer

Abnormal Regulation of Genes

Overexpression

Up-regulation

Down-regulation

Abnormal Signal Transduction

Abnormal Cell Surface Receptors

Drug Resistance Mechanisms

Gene Therapy/Reporter Genes

Molecular Targets in Oncology

- Signal Transduction
 - PI 3-Kinase
- Cell Cycle
 - Cyclins
 - Checkpoint Control Pathways (p53, G2-M)
 - Cyclin-dependent Kinases (Cdk)
 - Cdk Inhibitors (p16, p21)
- Multidrug Resistance
 - MDR1* P-glycoprotein
 - MRP
 - LRP
 - Topoisomerase

Molecular Targets in Oncology

- Angiogenesis
- p53
- Retinoblastoma tumor suppressor protein (RB)
- Apoptosis
 - Bcl-2
 - Bcl-x
- Extracellular matrix
- Immune response
- Telomerase

Molecular Imaging

- Monitoring Endogenous Gene Expression *In Vivo*
 - Gene (DNA), message (RNA), protein, function
 - Conventional approach to imaging receptors, enzymes, transporters (Octreoscan, FDG PET, ^{131}I)
- Monitoring Exogenous Gene Expression *In Vivo*
 - Gene (DNA), message (RNA), protein, function
 - Novel applications in basic and translational research as well as future clinical applications
 - » Gene therapy
 - Spatial and temporal heterogeneity of vector delivery and gene expression patterns
 - » Reporter genes

Potential of Molecular Imaging in the Clinic

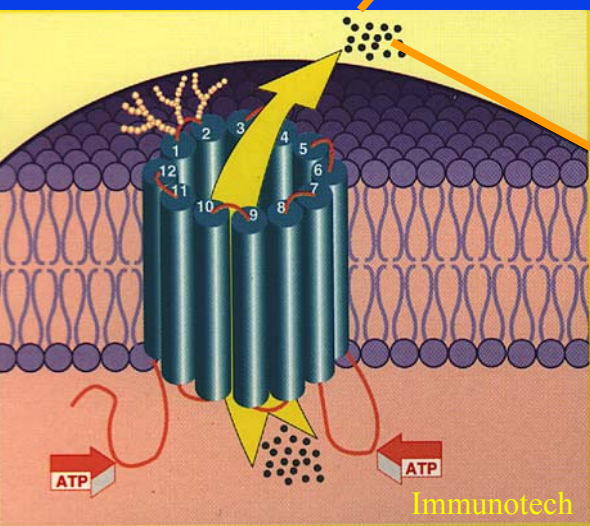
- **Non-invasive assessment of gene expression**
- **Early detection of disease**
- **Guide therapeutic choices**
- **Monitor drug action**
- **Aid pre-clinical drug development**
- **Non-invasively and repetitively monitor gene therapy and vector delivery**

General Strategies for Molecular Imaging

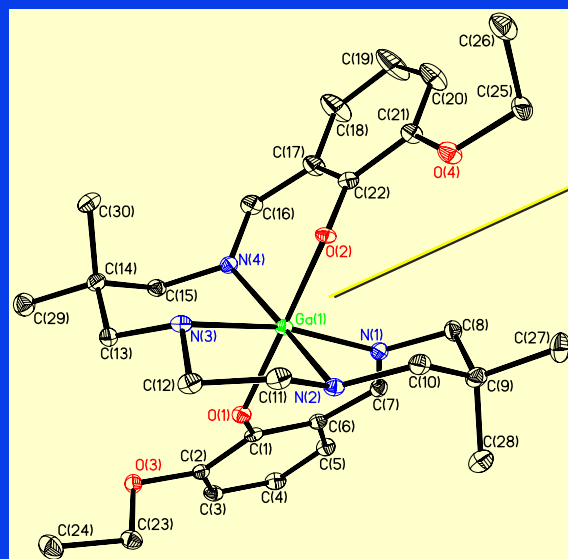
A main focus of molecular imaging is on validation and use of image enhancing agents that interact specifically with the molecular environment of cells

- Signal generation by transport, binding, or sequestration of a labeled reagent
- Signal amplification by an enzymatic activity acting on a reagent
- Reporter gene acting on a reporter probe

Molecular Imaging of Cancer Drug Resistance In Vivo: Imaging Multidrug Resistance (*MDR1*) P-glycoprotein (Pgp) Transport Activity *In Vivo* with PET and SPECT



mdr1 Pgp

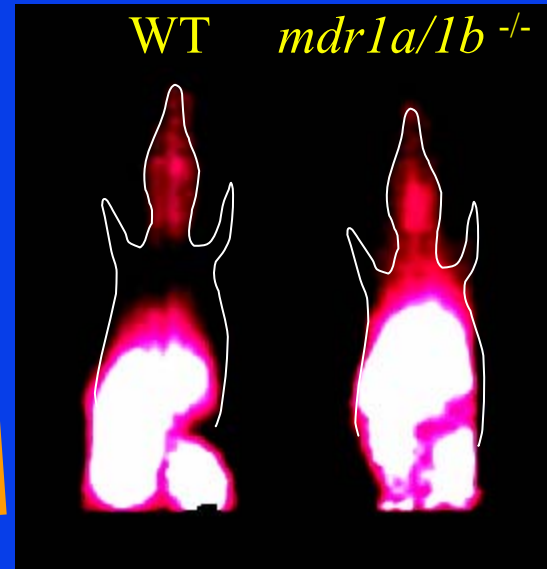


$[^{68}\text{Ga}]$ ENBPI Complex
(PET Radiopharmaceutical)

β^+

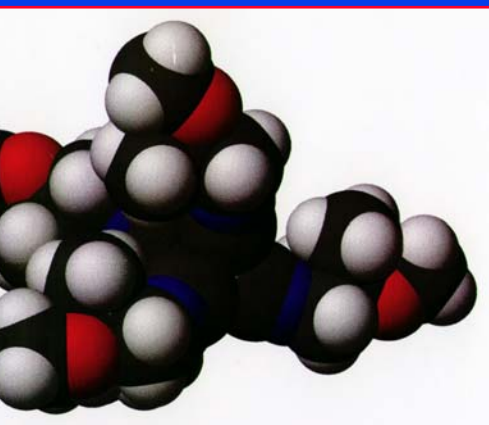
MicroPET

WT *mdr1a/1b*^{-/-}



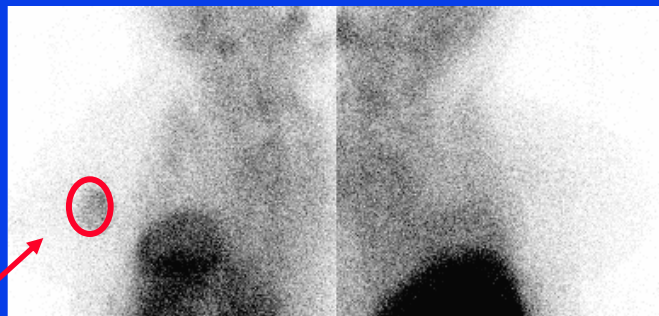
Coronal 10 min p.i.

New Applications with Established Radiopharmaceuticals: Molecular Imaging of *MDR1* P-glycoprotein Transport Activity In Breast Cancer with [^{99m}Tc]-Sestamibi

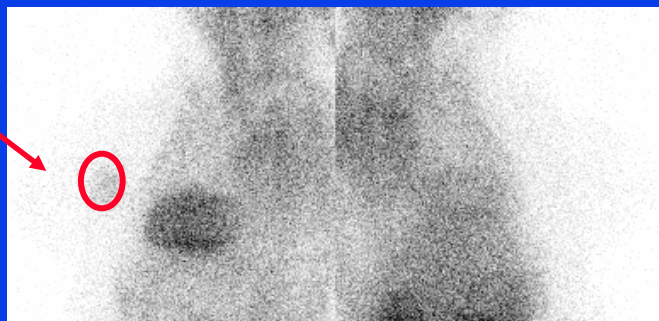


[^{99m}Tc]-Sestamibi

1° Tumor

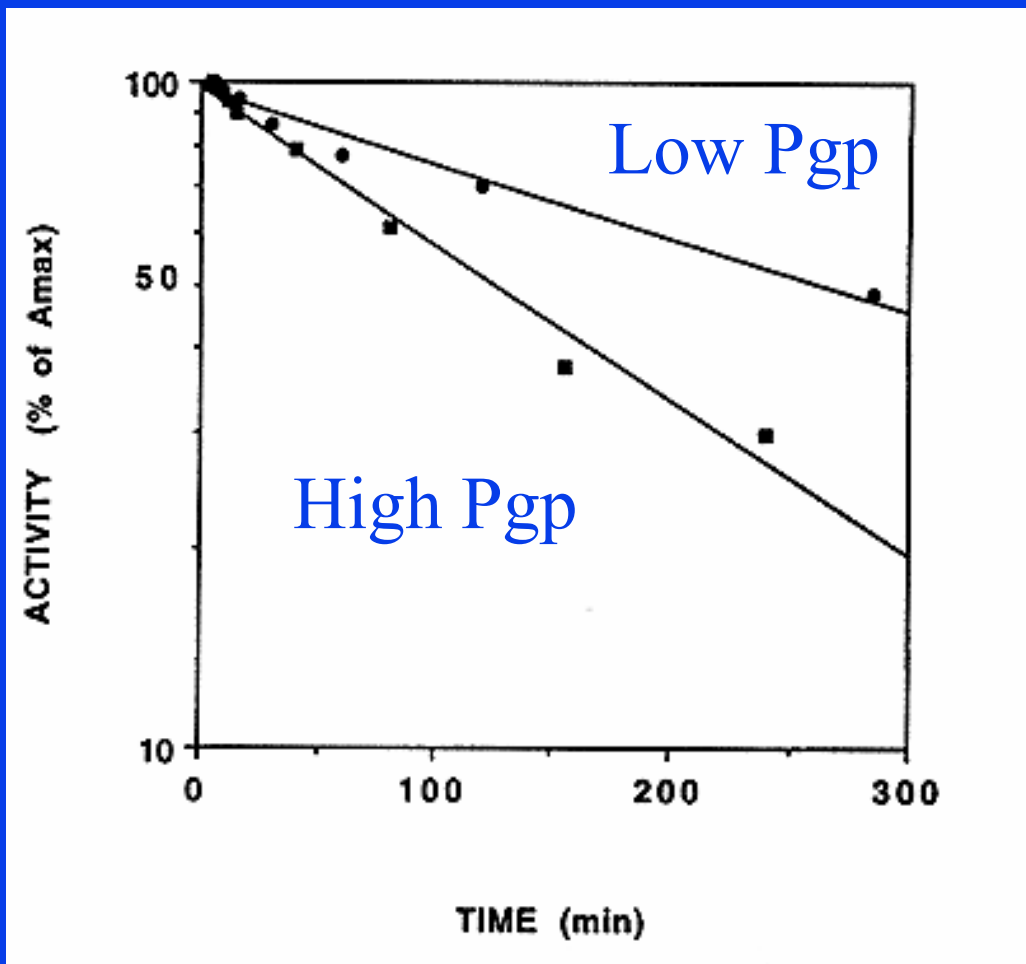


1 hr post-injection



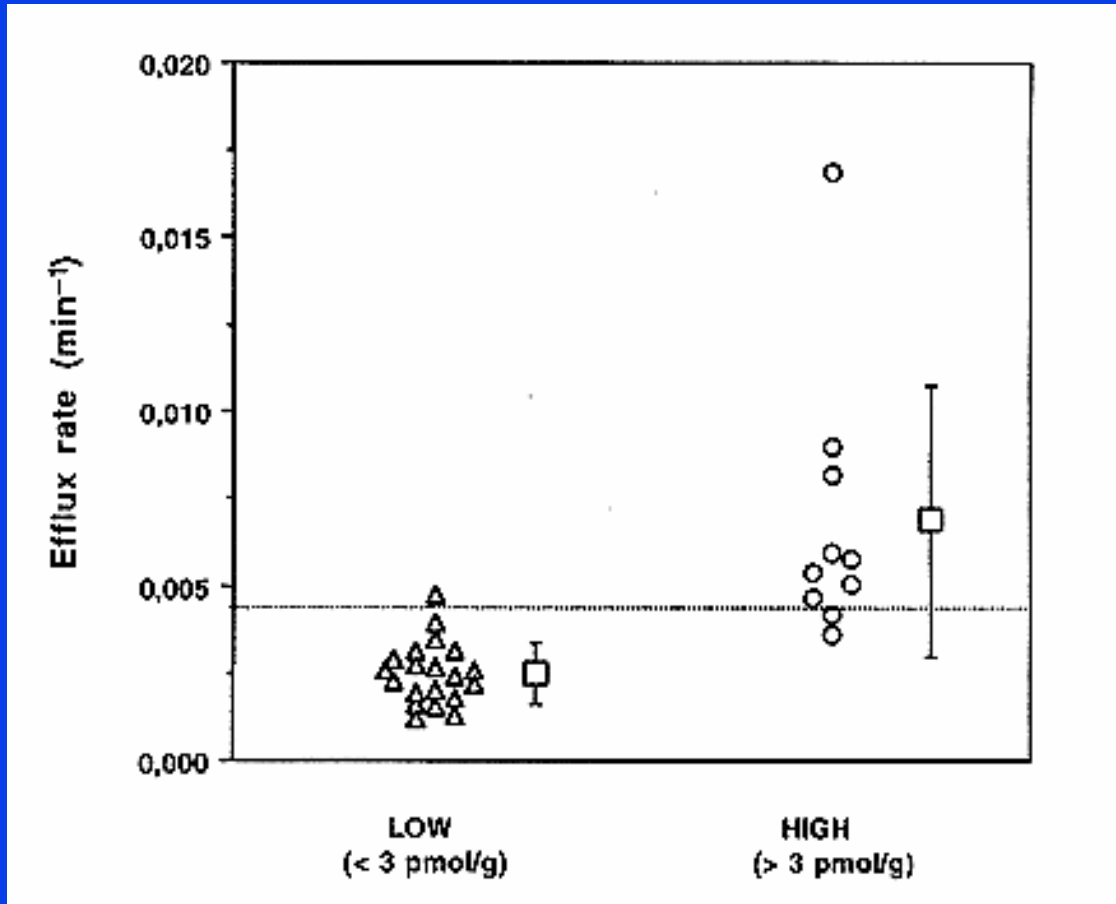
4 hr post-injection

Parametric Analysis of Images: ^{99m}Tc -Sestamibi Clearance From Breast Cancers *In Vivo*



Del Vecchio, et al, Eur J Nucl Med 24:150, 1997

Correlation of Tc-99m-Sestamibi Efflux Rate Constants and *MDR1* P-glycoprotein Expression in Breast Cancer



Del Vecchio, et al, Eur J Nucl Med 24:150, 1997

Vectors and Vector Delivery

Gene Therapy and Drug Delivery

- Viral Vectors (adenovirus, lentivirus)

- Non-Viral Vectors

- PEG co-polymers

- Naked DNA, RNAi

- Liposomes

- Membrane permeation peptides

 - Tat basic domain

- Cationic detergents

Structure for a $^{99\text{m}}\text{Tc}$ -Tat Peptide Complex

Tat peptide 48-57

Linker

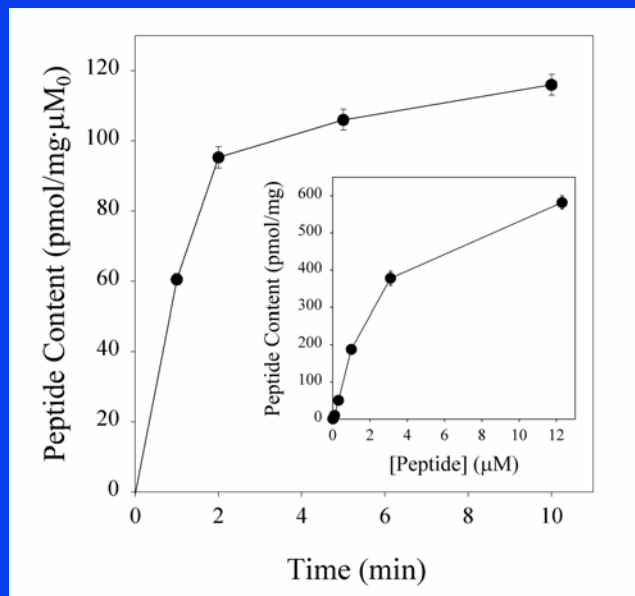
Chelate



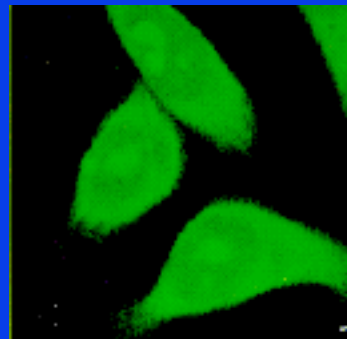
Molecular Imaging: Technetium-99mTat Peptide Conjugate

Bioconjugate Chem 11: 762-771, 2000

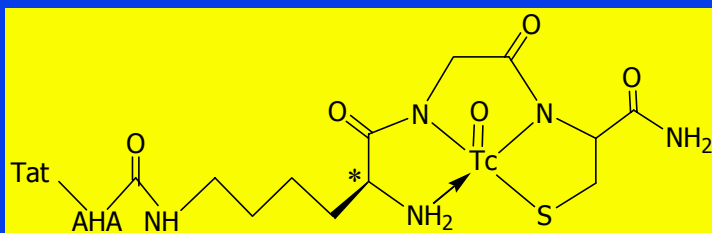
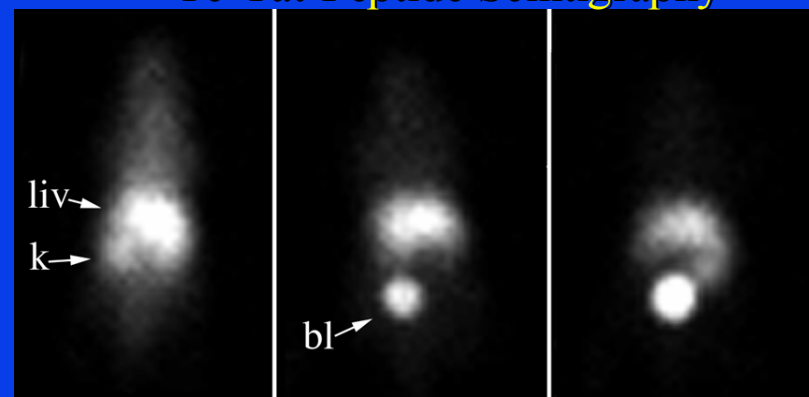
Uptake of ^{99m}Tc -Tat-Peptide in Jurkat Cells



Fluorescein-Tagged Tat Peptide in KB-3-1 Tumor Cells



^{99m}Tc -Tat-Peptide Scintigraphy



^{99m}Tc -Tat Peptide Complex

5 min 30 min 60 min
Mouse Post i.v. Injection

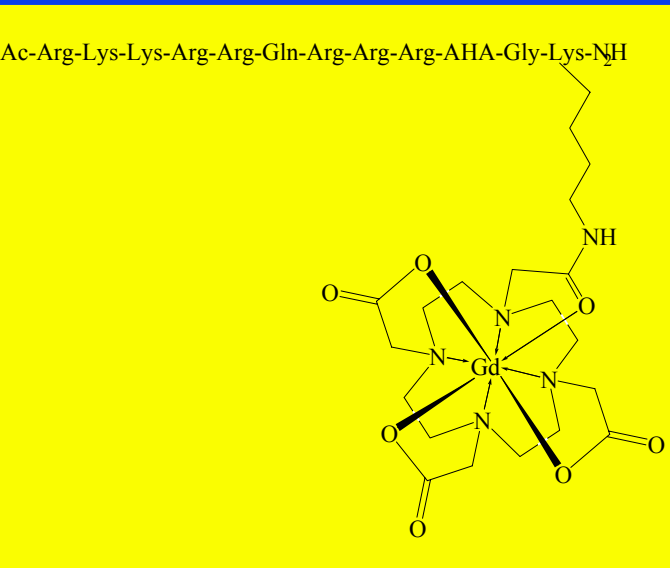
Platform Scaffold for Multimodality Membrane Permeant Imaging Agent: -weighted SE MRI of Human Jurkat Cells Pre-Treated with Gd-DOTA-D-7

Outer ring = Agarose

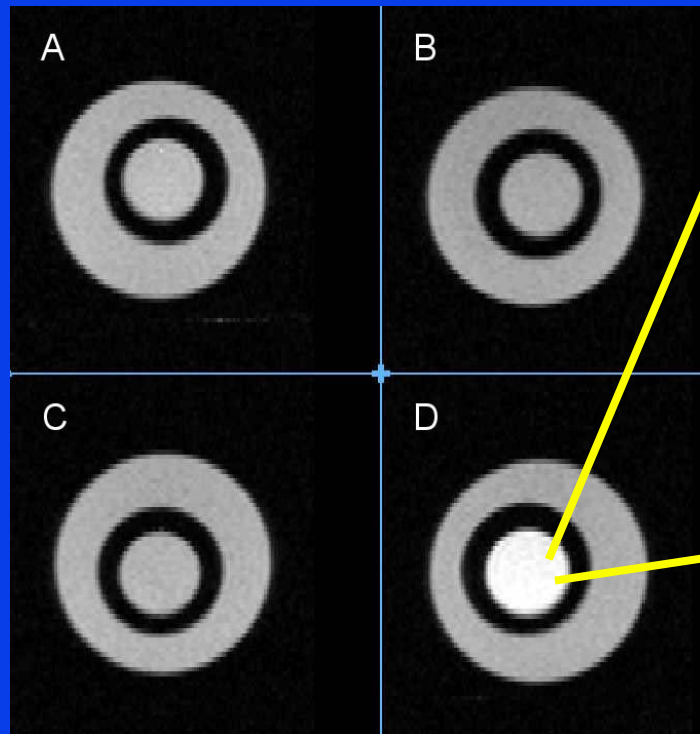
Inner ring

Agarose

Jurkat cells

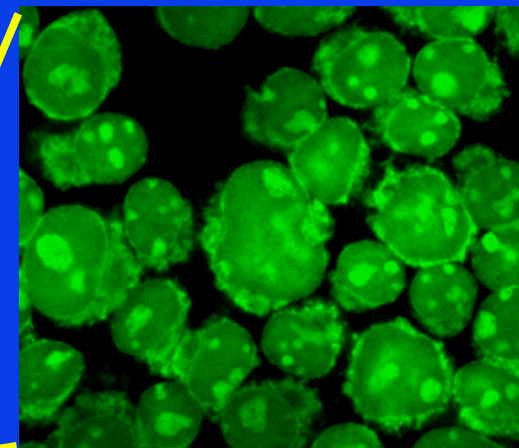


Gd-DOTA-D-Tat



Jurkat cells +
Omniscan

Jurkat cells +
Gd-DOTA-D-Tat



Jurkat cells +
Fluorescein-D-Ta

Molecular Imaging

- **Monitoring Gene Expression *In Vivo***

- **Concept of Reporter Gene**

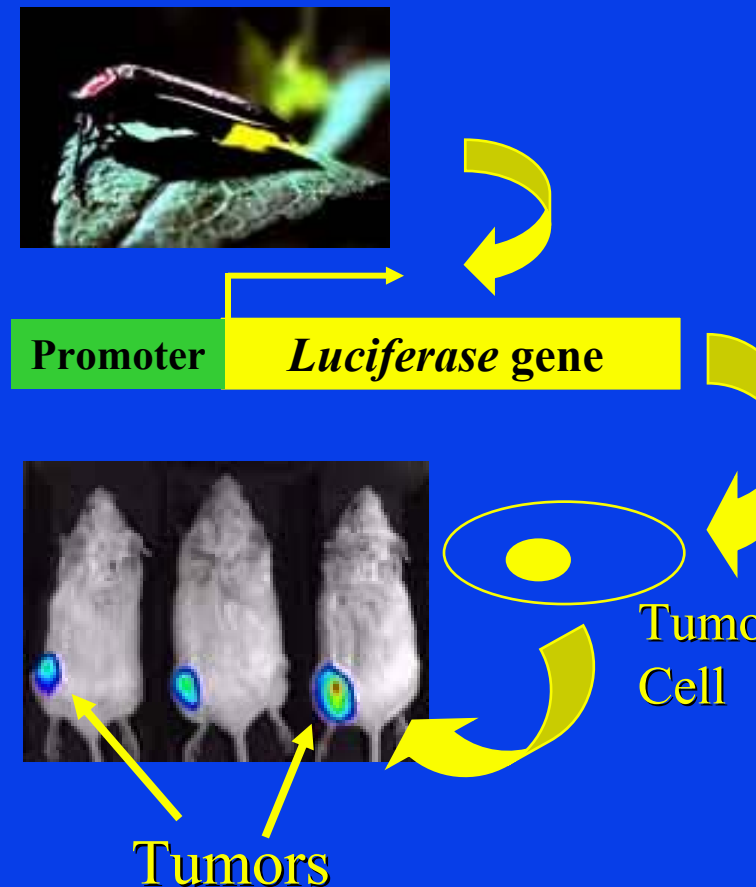
- Use a constitutive (always on) exogenous promoter to drive a reporter gene for labeling cells or tissues (CMV promoter – reporter gene)
 - Use to study regulation of an endogenous promoter with a reporter gene (MDR1 promoter – reporter gene)
 - Use to engineer gene deletion and transgenic mouse models

Molecular Imaging with Reporters

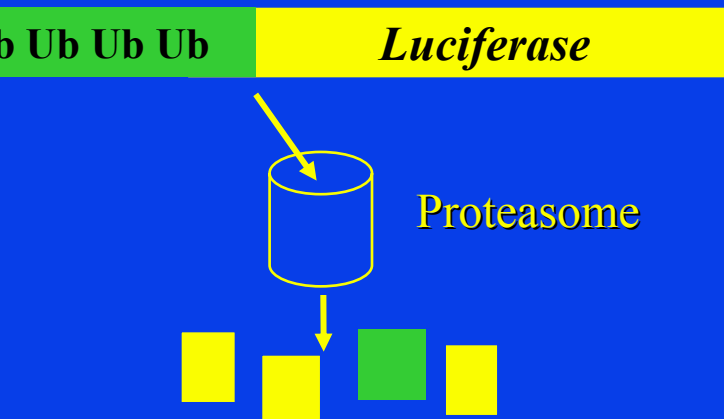
Bioluminescence Imaging *In Vivo* with Firefly Luciferase Reporter Genes



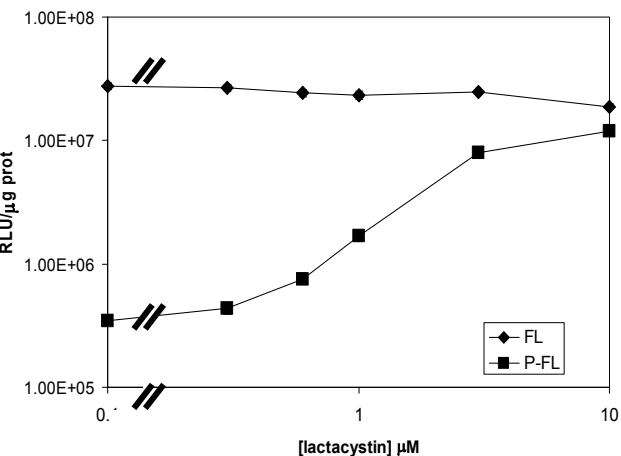
Xenogen



Noninvasive Bioluminescence Imaging of 26S Proteasome Function with a Ubiquitin-Luciferase Fusion Reporter Stably Expressed in Hela Cells Before and After Treatment with the Proteasome Inhibitor Lactacystin

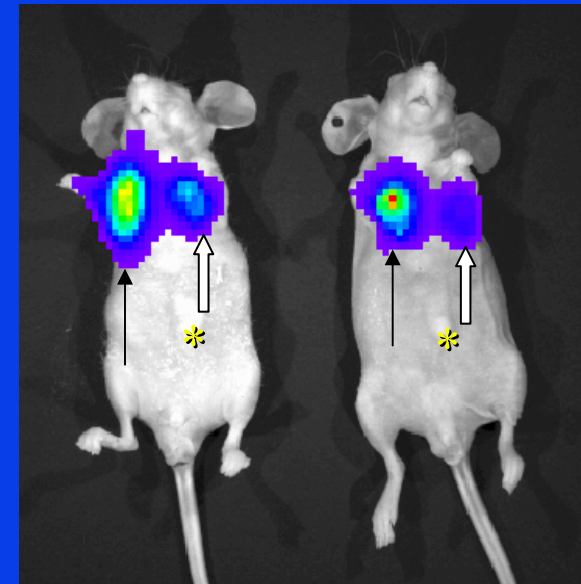


Dose-response with Lactacystin in Stable Transfectants



Control

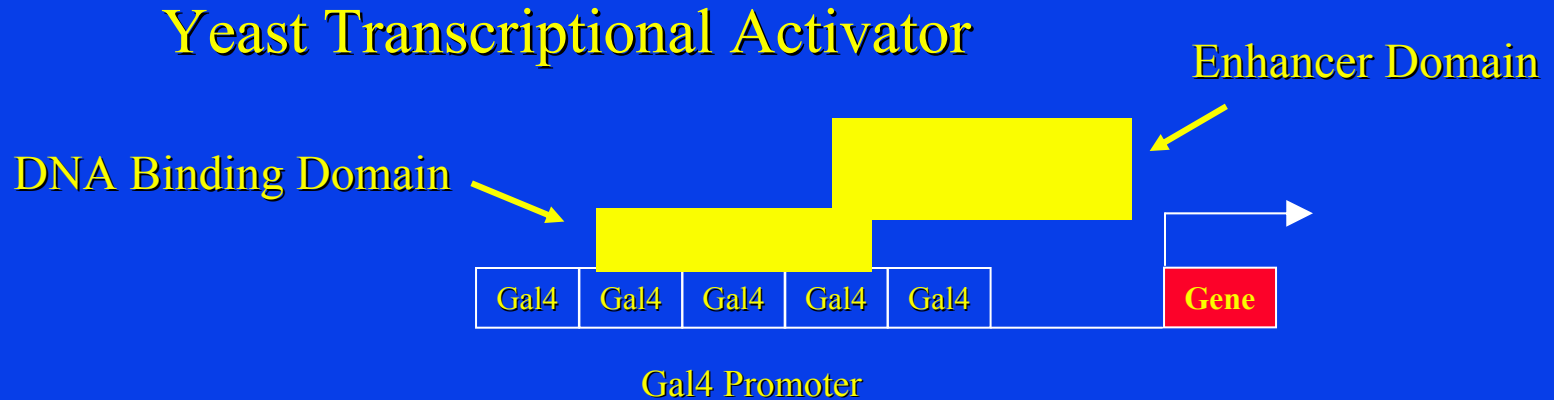
↑ Ub-Luc



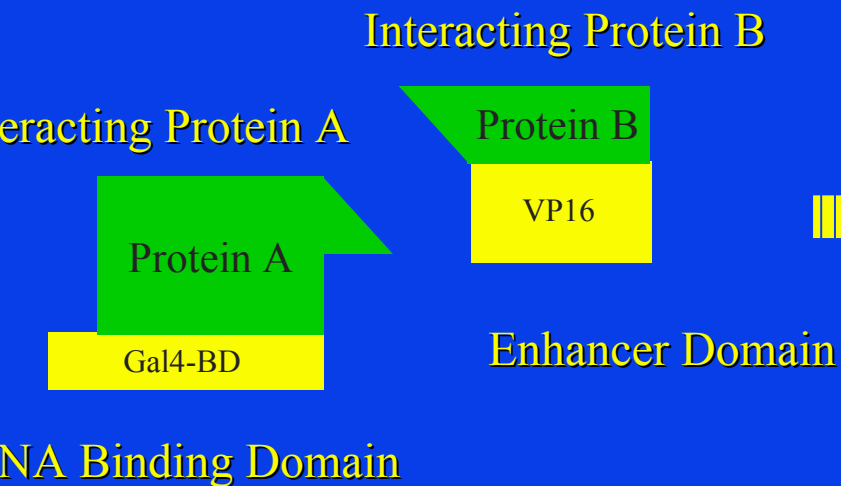
+ Lactacystin

↑ Luc * Vector

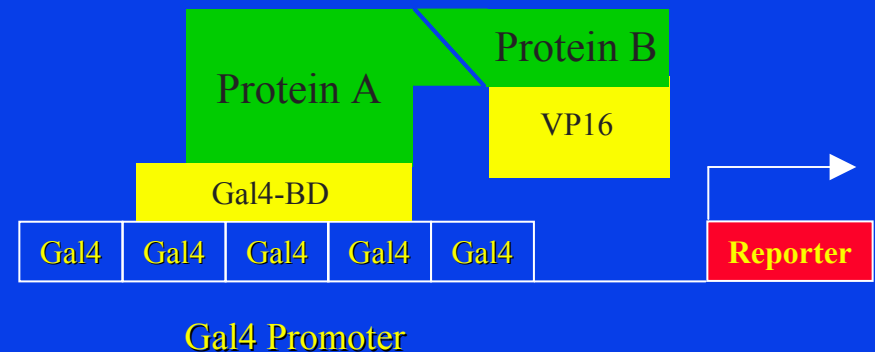
Example of Molecular Imaging Pre-Clinical Science: Analysis of Protein-Protein Interactions with Two-Hybrid Technology



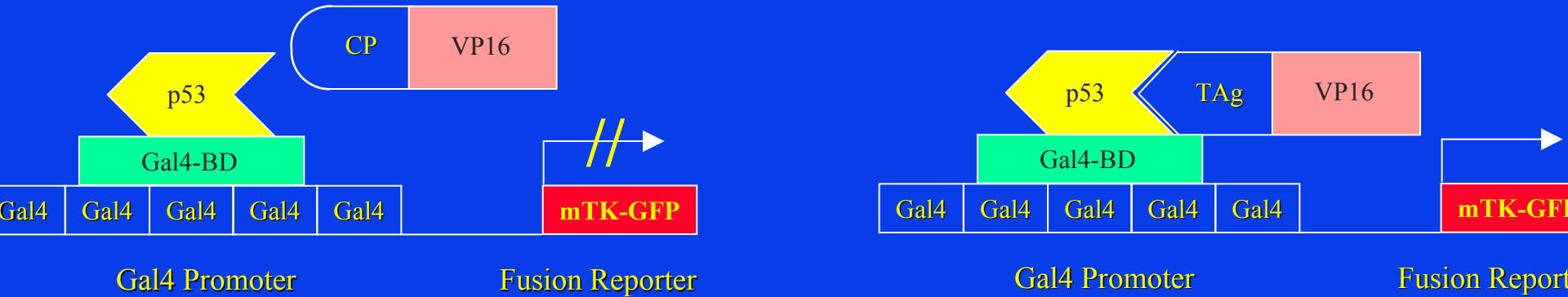
Hybrid Proteins



Protein-Protein Interactions



MicroPET Imaging of Protein-Protein Interactions in Living Mice: p53/Coat Protein (Negative Interaction) Versus p53/TAg (Positive Interaction)

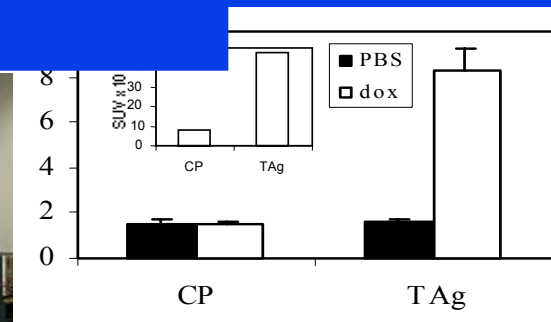


p53/CP
Hela Reporter Cells
Transfected with p53/CP

p53/TAg
cells
p53/TAg



Photo



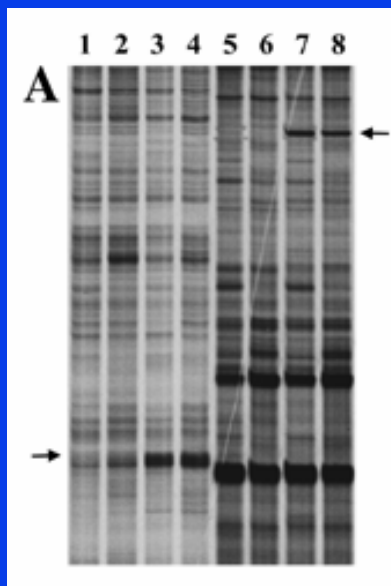
**Quantitative MicroPET
and Biodistribution Analysis**

Molecular Imaging and Molecular Medicine: Predicting the Future

- Conventional radiology has focused largely on the anatomic basis of disease
- Now that a first draft of the human genome project is complete and this information is being translated into medical practice, more and more disease will be diagnosed and treated before there is any evidence of anatomic change
- For many diseases, conventional radiology may become largely obsolete
- Molecular imaging will interface with future developments in molecular diagnosis and therapy

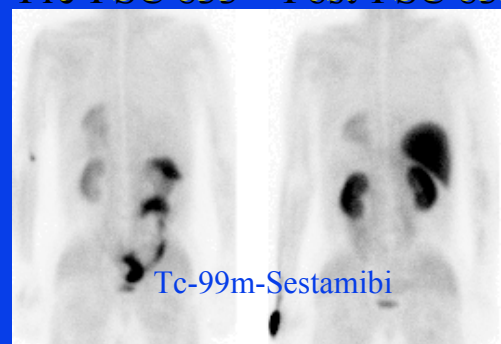
Molecular Imaging

Targeted Agents Link Cell Biology
to Imaging Technologies *In Vivo*



Imaging Reversal of P-glycoprotein *In Vivo*

Pre-PSC 833 Post-PSC 833



Action Plan: Invest in the discovery, validation and clinical approval process
of molecular targeted imaging agents that spatially and
temporally map molecular and cellular pathobiology *in vivo*

Research Needs for the Future

- To intercept trends in molecular medicine and genomics research, NIBIB should support and foster all aspects of molecular imaging (detectors, imaging agents, models systems) directed at interrogating disease processes at the cellular and molecular levels.
- Discovery, validation and development of platform image enhancing agents and multimodality imaging strategies as the signal transducers linking biology *in vivo* with imaging instrumentation.

Research Needs for the Future

- Fund hypothesis-driven research into fundamental pathways of cellular processes that cross diseases and organ systems.
- Training programs in molecular imaging that integrate concepts and methodologies of chemistry and cell/molecular biology.